



**Service of Biosafety and Biotechnology**

**Dr. W. Moens**

O./ref. : IPH/1520/SBB/03-0409

Y./ref. :

**Report on the molecular characterisation of the genetic map of event Mon810**

**16 June 2003**

## Table of Contents

<b>1. Introduction: Mon810 maize dossier.....</b>	<b>2</b>
<b>2. Overview of molecular data of event Mon810.....</b>	<b>2</b>
<b>2.1. Plasmid used for transformation.....</b>	<b>2</b>
<b>2.2. Characterisation of the insert and flanking regions .....</b>	<b>2</b>
<b>2.3. Comparison of molecular data of Mon810 provided in dossier C/F/95/12-02 with         data from CLO .....</b>	<b>3</b>
<b>3. Conclusions .....</b>	<b>3</b>
<b>4. References .....</b>	<b>3</b>
<b>5. Confidential information .....</b>	<b>4</b>

## 1. Introduction: Mon810 maize dossier

Mon810 notification C/F/95/12-02 of Monsanto is approved under Directive 90/220/EEC for growing, import, seed production and processing into animal feeding stuffs and industrial purposes since 22 April 1998 (Commission Decision 98/294/EC). In December 1997, food and food ingredients derived from Mon810 maize were notified under Article 5 of the Regulation (EC) 258/97.

Several hybrids of Mon810 are still pending for approval for marketing:

- T25 x Mon810 notification C/NL/98/08 was submitted under Directive 90/220/EEC on 29 April 1999. The Scientific Committee gave a favourable opinion on this dossier on 6 June, 2000.
- Mon810 x NK603 notification C/GB/02/M3/03 was submitted under Directive 2001/18/EC on 15 January 2003 for import and use in feed and industrial processing.
- Mon863 x Mon810 notification C/DE/02/09 was submitted under Directive 2001/18/EC on 7 February 2003 for import and use of grain and grain products. On 29 August 2002, the application was submitted under Regulation (EC) 258/97.
- MaisGard<sup>®</sup>/RR<sup>®</sup> (maize derived from Mon810 and GA21) notification C/ES/99/02 of Monsanto was submitted under Directive 2001/18/EC on 13 February 2003 for import and use in feed and industrial processing. On 16 March 2000, the maize application was submitted under Regulation (EC) 258/97.

The molecular data of the notification C/F/95/12-02 have been discussed during the meeting of the Belgium Biosafety Advisory Council on 6 December 2002. An overview of the molecular data of the event Mon810 presented during this meeting and provided by the applicant and CLO (Centrum Landbouwkundig Onderzoek, Melle, Belgium), is given below.

## 2. Overview of molecular data of event Mon810

### 2.1. Plasmid used for transformation

The maize line MON810 was produced by transforming the maize genotype Hi-II with two plasmid vectors, pV-ZMBK07 and pV-ZMGT10. The plasmid pV-ZMBK07 (see fig.1) contains the *cryIA(b)* gene, isolated from *Bacillus thuringiensis* ssp. *kurstaki*, is under the control of the enhanced cauliflower mosaic virus (CaMV) 35S transcription promoter (e35S) and the 3' nopaline synthase (nos) region of *Agrobacterium tumefaciens*. An intron from the maize heat-shock protein (*hsp70*) is located between the e35S promoter and the *cryIA(b)* gene. The second plasmid pV-ZMGT10 (see fig. 2) contains the CP4 EPSPS gene isolated from *Agrobacterium* strain CP4 and the *gox* gene cloned from *Achromobacter* strain LBAA. Both plasmids contain the *nptII* gene under control of a bacterial promoter.

### 2.2. Characterisation of the insert and flanking regions

According to the dossier, line Mon810 contains a single copy of the e35S promoter, the *hsp70* intron and the *cryIA(b)* gene (see annex 1). The absence of the 3' end of the NOS terminator

sequence was confirmed by the CLO (see annex 2). Molecular analysis performed by the applicant shows that the *nptII* gene and the backbone sequences of pV-ZMBK07 are not integrated and that none of the DNA sequences from vector pV-ZMGT10 are present in line Mon810 (see annex 1).

The sequence of the 5' junction, upstream from the CaMV e35S transcription promoter was determined by the CLO. The DNA shows 88% identity with the 22 kDa alpha zein gene (see annex 2). The sequence at the junction with the e35S promoter has been obtained independently by Holck *et al.* (2002) and is in agreement with that obtained by the CLO. This junction sequence has been submitted to the EMBL sequence database (EMBL accession n° AF434709).

In conclusion, the Mon810 line contains a 3' truncated *cryIA(b)* cassette. The exact 3' limit of this gene and the adjacent host DNA is unknown. The 5' junction corresponds to the 5' end of the CaMV e35S promoter and the host alpha zein gene.

### 2.3. Comparison of molecular data of Mon810 provided in dossier C/F/95/12-02 with data from CLO

Table 1: Comparison of molecular data of event Mon810 according to dossier with data provided by CLO

Dossier <sup>#</sup>	CLO*	Remarks
1 transgene insert: pe35S- <i>hsp70</i> intron- <i>cryIA(b)</i>  3': nos truncated  5': no flanking plant DNA sequences analysed	Confirmation of 3'nos truncation  5': about 100 bp plant DNA analysed = corn DNA (zein)	No flanking plant DNA sequences reported at 3' end of the transgene

<sup>#</sup> see annex 1

\* see annex 2

### 3. Conclusions

The molecular data presented in the dossier C/F/95/12-02 do not completely fulfil the Belgian requirements concerning molecular data. The sequences of the flanking regions are not provided in the dossier, so rearrangements at these sides cannot be excluded. In addition, bio-informatic analysis should be done to determine the presence of chimaeric open reading frames in the border integration sequences.

### 4. References

Holck, A., Vařtilingom, M., Didierjean, L. and Rudi, K. (2002) 5'-nuclease PCR for quantitative event specific detection of the genetically modified Mon810 Maisgard maize. *European Food Research and Technology*, **214**, 449-453.

**Acknowledgements.** We would like to thank the experts of the working group 'Molecular Characterisation' of the SBB to contribute to this report.

## **5. Confidential information**

List of figures:

Figure 1: Plasmid map of pV-ZMBK07

Figure 2: Plasmid map of pV-ZMGT10

List of annexes:

Annex 1: Molecular data from dossier C/F/95/12-02

Annex 2: CLO Report p.8-9